REMARKS

Status of Claims

Claims 1, 2, 5-21 and 23-30 are pending. Claims 10-19 and 28-30 are withdrawn from consideration. Claims 1-2, 5-9, 20-21 and 23-27 have been rejected.

Claims 1, 2, 10-14, 19-21, 23, 29 and 30 have been amended. Amendments to claims 2, 10-14, 19, 21, 23, 29 and 30 are clerical in nature. Amendments to claims 1 and 20 are clerical and/or supported throughout the application and claims as filed. Applicants assert that no new matter has been introduced in the claim amendments.

Claims 6 and 24 have been canceled without prejudice or disclaimer. In making this cancellation without prejudice, Applicants reserve all rights in these claims to file divisional and/or continuation patent applications

CLAIM REJECTIONS

35 U.S.C. § 112 Rejections

In the Office Action, the Examiner rejected claims 1-2, 5-9, 20-21 and 23-27 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

In the Office Action, the Examiner alleged that the specification does not describe bacterial vaccine vectors with stabilized virulence factors following the second passage in that a) Figures 1 and 2 allegedly show maximum load after the third passage (passage 2) using the definition of passages from paragraph [0087] of the published specification and b) Figure 1 shows a leveling off of bacterial load rather than a peak in bacterial load as allegedly described in paragraph [0087] of the published specification.

First, it can be seen in Figure 1 that maximum load is reached after passage 2. Second, the claim does not refer to a "second passage". Finally, a skilled artisan would understand that maximum load would be either a leveling of virulence as shown in Figure 1 and

described in paragraph [0145] of the published application or a peak of virulence as described in paragraph [0087]. Applicants therefore request withdrawal of the rejection.

In the Office Action, the Examiner rejected claims 1-2, 5-9, 20-21 and 23-27 under 35 U.S.C. § 112, second paragraph, as allegedly being incomplete for omitting essential elements. The Examiner alleged that Applicants need to include a) the essential element that stabilizes virulence following the second passage of the vaccine vector and b) the essential element that links expression of the heterologous antigen with stabilized virulence.

Specifically, the Examiner alleged that it is not clear how virulence induced by passaging will remain stable indefinitely following the second passage. Applicants point out that the claimed method may be used to produce a bacterial vaccine vector with stabilized virulence that is ready for use and/or preservation, for e.g., in a master cell bank (MCB) to create a commercial product which is then preserved such that no further bacterial generations occur until the agent is administered. The method is therefore useful as claimed.

Further, regarding the essential element that stabilizes virulence following the second passage of the vaccine vector, Applicants point out that ("[an] inventor need not understand precisely why his invention works in order to achieve an actual reduction to practice"). *Parker v. Frilette*, 462 F.2d 544, 547, 174 USPQ 321, 324 (CCPA 1972) (MPEP 2138.05).

Regarding the essential element that links expression of the heterologous antigen with stabilized virulence, the claimed method describes specific steps to enhance the immunogenicity of a bacterial vaccine vector expressing a heterologous antigen. Applicants have demonstrated that the claimed method is functional for enhancing immunogenicity using three separate heterologous antigen expression systems (see Figures 1-3 and Example 1). Therefore, while the location, timing, and nature of antigen expression may or may not be relevant to the maximum bacterial load that may be reached (as measured by PFU, for e.g.), the claimed method may be used to bring the vector to its maximum bacterial load and stabilize its virulence, regardless of the identity and properties of the heterologous antigen. Thus, the method steps including passaging links to stabilized virulence regardless of the heterologous antigen. Applicants therefore request withdrawal of the rejection.

35 U.S.C. § 102 Rejections

In the Office Action, the Examiner maintained his rejection of claims 1-2, 5-9, 20-21 and 23-27 under 35 USC 102(e) as allegedly being anticipated by Pawelek et al. (US Patent No. 6,685,935).

Claims are directed to: *A method of enhancing the immunogenicity* of a Listeria vaccine vector *expressing a heterologous antigen* or of an antigen expressed from a Listeria vaccine vector, the method comprising the steps of: a) administering to an animal the Listeria vaccine vector; b) passaging the Listeria vaccine vector through the animal; c) harvesting the Listeria vaccine vector from the animal, and; d) repeating step a), step b), and step c) with the harvested bacterial vaccine vector *until a maximum bacterial load for said vector in an organ is reached* and *virulence is stabilized*, thereby *enhancing the immunogenicity of the bacterial vaccine vector.*

Contrary to the Examiner's allegations, Pawelek does not describe a) enhanced immunogenicity after in vivo passaging; b) a maximum bacterial load or stabilized virulence of the vector; or c) passaging of a vector expressing a heterologous antigen and therefore does not anticipate the claimed invention.

The Examiner alleged that Pawelek passages a bacterial vaccine vector through an animal resulting in enhanced immunogenicity associated with endotoxin release. Applicants disagree.

Contrary to the Examiner's allegation, Pawelek does not demonstrate immunogenicity associated with endotoxin release and certainly not an enhancement of immunogenicity to endotoxins as a result of vector passaging. First, there is no measurement of the expression of endotoxins in passaged vectors. In fact, Pawelek describes deletion of LPS (col 26, lines 22-37) or of the genes involved in synthesizing endotoxin (col 66 last paragraph – col 67 first paragraph) to avoid sepsis. Pawelek therefore teaches away from using a Salmonella expressing an endotoxin.

Second, in Salmonella, LPS is endogenous and not a heterologous antigen, as claimed, and no mention is made of using LPS as a heterologous antigen in another species.

Third, although Salmonella inherently elicits an immune response, Pawelek does not describe *enhancing* the immune response by passaging the vector, but instead describes enhancing tumor specificity (see abstract). Pawelek has no experiments directed to the ability of the passaged vectors to elicit an immune response. Pawelek therefore does not describe a method of enhancing immunogenicity comprising the claimed steps.

In addition, the Examiner alleged that Pawelek demonstrated enhanced virulence after in vivo passaging. Applicants claim stabilized rather than enhanced virulence, and Pawelek does not demonstrate stabilized virulence after passaging.

Pawelek also does not describe passaging until a maximum bacterial load in an organ is reached. In Pawelek's method, the bacterial load for the tumor is decreased in that each passage is more stringent than the prior passage (fewer inoculated bacteria, less infection time) (Pawelek, col. 37, para 1; Table 9). Similarly, Pawelek does not describe stabilized virulence after passaging, in that the number of bacteria recovered in tumors was variable. The Examiner alleged that Pawelek's mouse-passaged bacteria were present at a maximum level $10x10^{6or7}$. Although $10x10^{6or7}$ may represent the maximum level of bacteria measured by Pawelek, it in no way represents the maximum bacterial load after passaging, as claimed. Pawelek never repeats a single passaging protocol until virulence is stabilized. Instead, with a different goal in mind, Pawelek's Table 9 presents the levels of bacteria that were in the tumor after each passage where the number of inoculated bacteria and the infection time varied.

Finally, Pawelek does not describe passaging a bacterial vaccine vector expressing a heterologous antigen. Pawelek describes that vectors are first passaged to enhance tumor specificity and then engineered to express for e.g., a suicide gene in the tumor to eradicate it (Section 6.2.1. and Example 13). Thus, Pawelek does not suggest passaging a vector expressing a heterologous antigen, as claimed.

Applicants respectfully request reconsideration and withdrawal of the rejections.

In the Office Action, the Examiner rejected claims 1, 2, 7-9, 20-21 and 25-27 under 35 USC 102(b) as allegedly being anticipated by Coulson et al. (Vaccine, 1994).

The Examiner alleged that Coulson discloses a method of passaging bacteria vaccine vectors resulting in increased maximum bacterial load (increased ability to colonize host tissues) and stabilized virulence (increase in colonization factors, rPA).

Applicants disagree. Coulson does not describe passaging until a maximum bacterial load in an organ is reached as is claimed. Coulson describes colonization of spleen tissue after a single passage to promote the stable expression of Anthrax Protective Antigen (PA). Coulson makes no mention of reaching a maximum bacterial load nor do they repeat the passaging so that it would be possible to discern if maximum bacterial load has been reached. Coulson does not describe stabilizing virulence, but rather stabilizing antigen expression. Coulson therefore does not anticipate the claimed invention.

The Examiner alleged that Coulson described reaching maximum load, but the Examiner brings as evidence only 1) that clone G3 (comprising PA) was able to colonize to the level similar to WT bacteria (not comprising PA) and 2) that G3 host carried the plasmid stably and colonized at "high" levels, neither of which demonstrates maximum bacterial load, which would require a controlled comparison of a single type of plasmid between passages until a maximum level of virulence or colonization is reached.

The Examiner similarly alleged that enhanced colonization ability is equivalent to stabilized virulence and that increased stability of a PA-expressing plasmid *in vivo* is also equivalent to stabilized virulence. Applicants disagree. The showing of an enhancement of colonization alone provides no evidence that further passaging would not further enhance colonization. Also, stabilized expression of anthrax PA protein in Salmonella is not equivalent to stability of virulence in that the plasmid expressing PA could be maintained in the host while the virulence may decrease, as commonly happens when growing bacterial generations in culture (see paragraph [0138] of the subject application).

The Examiner alleged in the office action on page 12 that Applicants claim an *increased* maximum bacterial load. The commonly understood definition of maximum is "The greatest possible quantity" (Merriam-Webster dictionary Online, downloaded April 29, 2010, **attached**) and so the phrase "increased maximum" is difficult to understand. Further, Applicants claim a maximum bacterial load rather than an *increased* maximum bacterial load. Coulson does not demonstrate a maximum of any kind, and does not observe the effect of

passaging on colonization of a particular strain, but in fact, compared colonization of G3 only to WT, where G3 comprises a plasmid and WT does not. There is no evidence that Coulson's WT Salmonella represents maximum bacterial load after passaging so that it may serve as a bench mark for maximum bacterial load for plasmid-containing Salmonella.

The Examiner also alleged that Applicants claim stabilized virulence factor expression, while in fact, Applicants claim stabilized virulence. The Examiner alleged that rPA (Baccilus anthraxis Protective Antigen) is a colonization factor, while there is no evidence in Coulson supporting that contention. Finally, the Examiner alleged that a showing that an increase in colonization factors anticipates the claimed invention, while the claims require stabilized virulence rather than enhanced or increased virulence.

Taken together, Coulson does not describe in vivo passaging of vector until a maximum bacterial load in an organ is reached or stabilized virulence is obtained. Applicants respectfully request reconsideration and withdrawal of the rejections.

Conclusion

In view of the foregoing amendments and remarks, Applicants assert that the pending claims are allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 50-3355.

Respectfully submitted,

/Mark S. Cohen/

Mark S. Cohen
Attorney/Agent for Applicant(s)
Registration No. 42,425

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Pearl Cohen Zedek Latzer, LLP
1500 Broadway, 12th Floor
New York, New York 10036
Tel: (646) 878-0800
Fax: (646) 878-0801